

# Phenol rich wastewater treatment using an aerobic granular sludge SBR

C. Bumbac, R. L. Dinu, A. M. Popescu, E. Pena-Leonte

National Research and Development Institute for Industrial Ecology – ECOIND,  
90-92 Panduri Street, 050663, Bucharest, sector 5, [biotehnologi@incdecoind.ro](mailto:biotehnologi@incdecoind.ro)

**Keywords:** phenol; SBR; wastewater treatment; aerobic granular sludge;

## Abstract

Phenol can be removed from wastewaters by biological treatment, which is generally preferred to physical or chemical treatment methods, because of lower costs and the possibility of complete mineralization. Many bacteria are capable of using aromatic compounds such as phenol as the sole source of both carbon and energy. While phenol wastewaters are usually treated in continuous activated sludge processes, these systems are known to be sensitive to high phenol loading rates and to fluctuations in phenol loading. Formation of microbial granules from activated sludge flocs, under aerobic conditions is currently an active area of investigation for developing new generation wastewater treatment plants for high strength organic wastewaters, bioremediation of toxic aromatic pollutants including phenol, toluene, pyridine and textile dyes, removal of nitrogen, phosphate and adsorption of heavy metals.

Aerobic granules have been successfully cultivated, previously, in SBR systems from flocculated activated sludge fed with acetate as the sole carbon source.

The main objective of this study was to investigate how phenol loading affected the structure and activity of aerobic granules. The results indicated that phenol loading exerted a profound influence on the structure and activity of the aerobic granules. Compact granules with good settling ability were maintained at loadings up to 3.0 kg phenol m<sup>-3</sup> day<sup>-1</sup>, and structurally weakened granules were observed at the highest loading of 3.7 kg phenol m<sup>-3</sup> day<sup>-1</sup>. Phenol-degrading aerobic granules possess high activity, compact structure and good settleability, and have the potential to treat wastewater with high phenol loading in sequential system at relatively low hydraulic retention times.

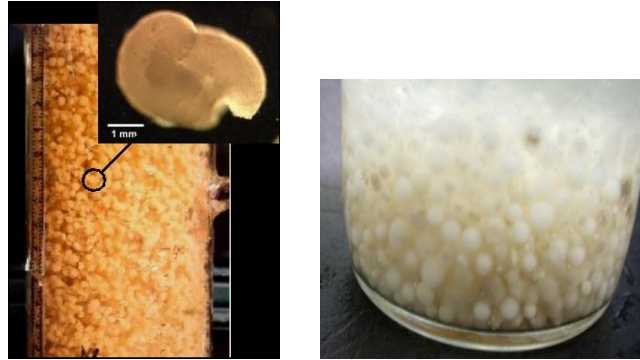
## Introduction

Phenol is a toxic and inhibitory substrate, but also a carbon source for the bacteria. The consequence of the presence of phenol in biological wastewater treatment is process instability, which can lead to the washout of the microorganisms (Allsop et al., 1993). In low concentrations, phenol is biodegradable, but high concentrations can kill phenol-degrading bacteria. Industrial wastewaters from fossil fuel refining, pharmaceutical and pesticide processing are the major sources of phenolic pollution.

At the end of the 1990s, based on the researches on biofilm structure and on the role of storage polymers (extracellular polymeric substances - EPS ) on biofilm formation lead to the idea of growing aerobic granules without carrier material on readily biodegradable substrates in Sequencing Batch Reactor (SBR) (van Loosdrecht, 1997).

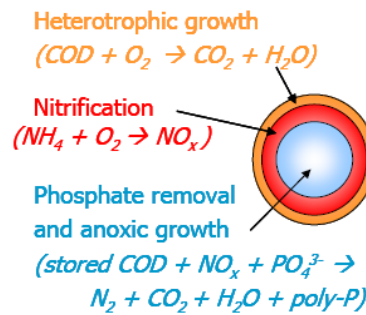
Microbial granules can be regarded as compact and dense microbial aggregates with a spherical structure fig.1 (Adav et al., 2008), each aerobic granule representing an enormous metropolis of microbes containing millions of individual bacteria.

The granular structure has many advantages. Due to diffusion gradients of substrate, nutrients and oxygen, the various process conditions usually accommodated in various tanks are now concentrated inside the granular sludge and thus only one tank is needed without large recycle flows to achieve similar efficiencies concerning organic load, nitrogen and phosphorus removal fig.2.



**Fig.1.** Typical granules aspect in laboratory scale reactors (Adav et al., 2008; de Kreuk, 2007)

Granular sludge is less susceptible to relatively high concentrations of toxic compounds in the influent because much of the biomass in the granules is not exposed to the same high concentration as present in the wastewater.



**Fig.2.** Schematic representation of different process conditions within the sludge granule

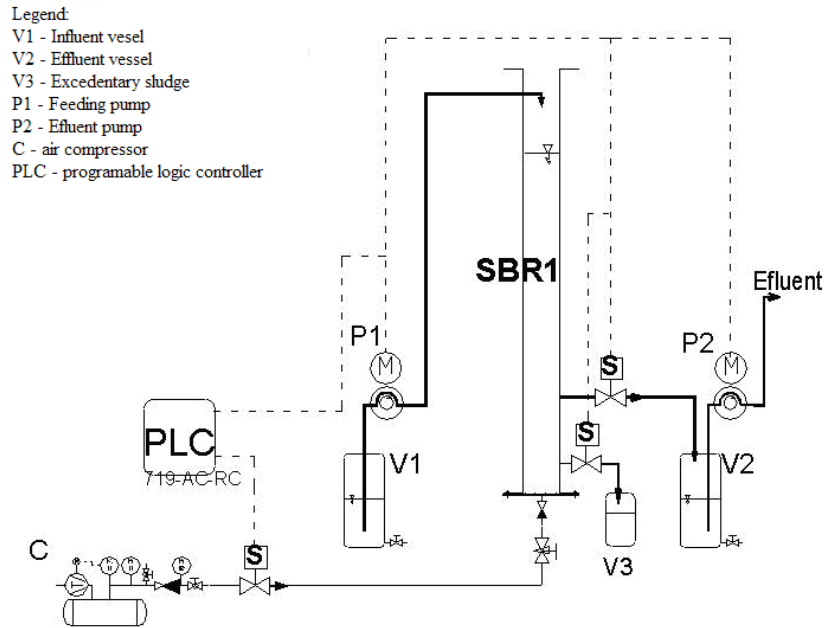
## Materials and methods

Aerobic granular sludge was successfully cultivated from conventional activated sludge as previously described by Bumbac et al. (2009) in a pilot-scale column-type sequencing batch reactor which had an internal diameter of 12 cm and a reactor height of 120 cm (fig.3). The cyclic operation of the SBR system was controlled by a PLC (programmable logic controller) using the following time sequence: 10 minutes influent feeding (VERDER peristaltic pump); 11,5 hours of aerobic reaction (5 L/min airflow velocity), 10 minutes of settling, 10 minutes of effluent withdrawal. The relative high airflow velocity was used in order to assure sufficient hydrodynamic shear forces needed for granule formation.

Settling time is an important hydraulic selection pressure operational parameter on the microbial community in the bioreactor. Thus, a short settling time preferentially selects for the growth of fast settling bacteria while the sludge with a poor settleability is washed out.

Synthetic wastewater (after a recipe adapted from Zhang et al. 2007), with sodium acetate as the sole carbon source and all macro and micro elements needed for bacterial growth/development, was used for the cultivation of aerobic granular sludge.

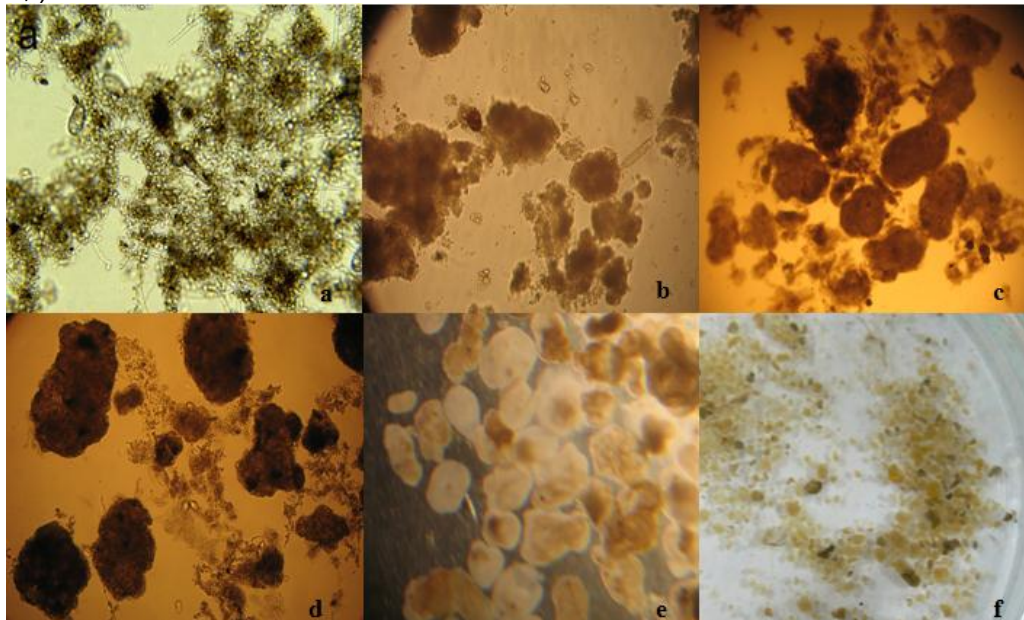
When predominant granular sludge was obtained, gradually increasing concentrations of phenol, in the range of 200 – 3500 mg/l were added to the synthetic wastewater to evaluate the stability and performance of the SBR system for high organic loads and relatively toxic substrates.



**Fig. 3.** Schematic representation of the aerobic granular sludge SBR

### Results and discussions

Predominant granular sludge with round shaped, compact granules of 1,5-3 mm in diameter was obtained within 60 days of operation of the acetate fed SBR. Figure 4 presents the evolution in time of aerobic granules formation process starting from the initial inoculum (conventional activated sludge from Pitesti WWTP) to bacterial agglomerations (fig.4b), predominant granular sludge (fig 4.c, d) and granular sludge (fig.4.e,f).



**Fig. 4.** Microscopic aspect of aerobic granules evolution from (a) seed sludge (microscopic image 20x), in time: (b)-after 20 days sludge agglomerations (microscopic image 20x); (c) after 40 days aerobic granules (microscopic image 10x); after 60 days predominant granular sludge: (d) microscopic image 4x; (e) stereomicroscopic image (4x); (f) digital camera photo.

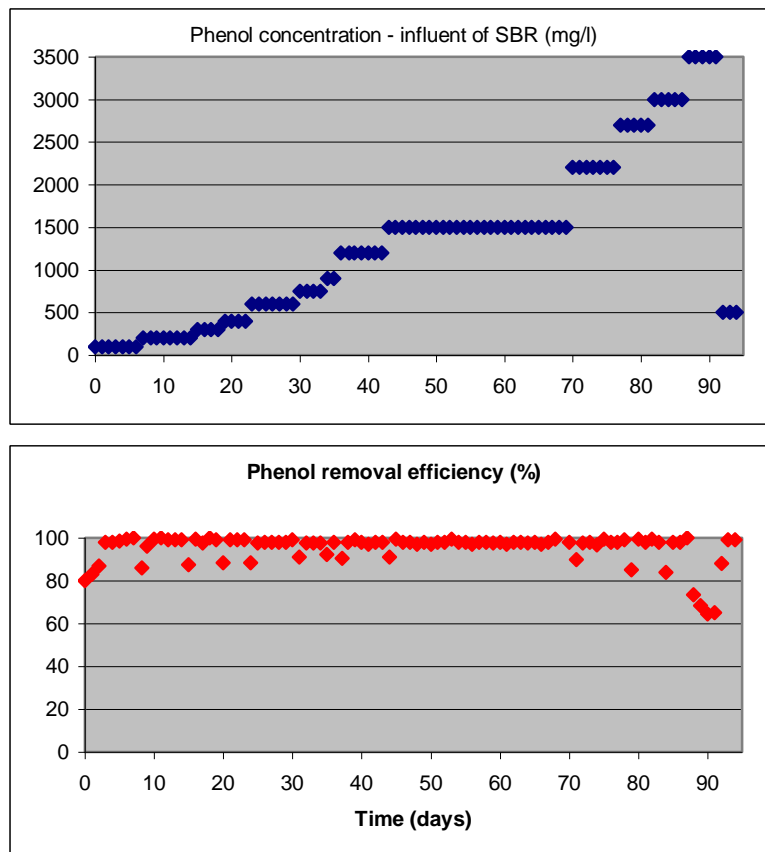
Aerobic granular SBR systems stability for high organic loads and relatively toxic substrates was assessed by adding gradually increasing phenol concentrations ranging

from 200 mg/l to 3500 mg/l in the synthetic wastewater. Stable, high rate phenol-degrading granules were developed within 2 months of operation. The compact structure of the acetate-fed granules likely protected the microorganisms against phenol toxicity and facilitated microbial acclimation towards higher phenol concentrations and faster phenol degradation rates as shown in fig.5. However, biomass washout and system failure have occurred at the highest concentration of 3.5 g/L, when the structural integrity of some of the granules was compromised because of phenol toxicity, thus forcing us to decrease the phenol concentration to 500 mg/L in order to recover the system performances.

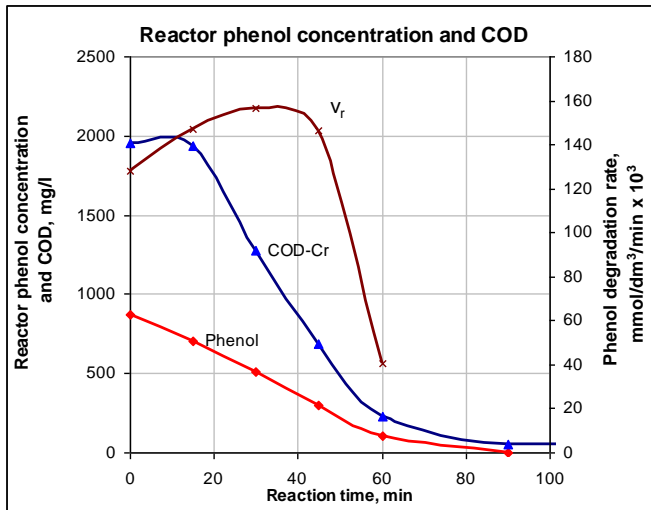
Aerobic granules exposed to phenol concentrations in the range of 100 to 3000 mg/L were able to exhibit good settling ability, up to 18m/h (fig.6) with good biomass retention and good metabolic activity (fig.5a and 5b).

As shown by the figure 6, the settling velocity is decreasing and also the settling pattern is changing with the increase of phenol feed concentration. After first ten settling minutes, no significant thickening of the sludge is observed (a major difference versus flocculent sludge).

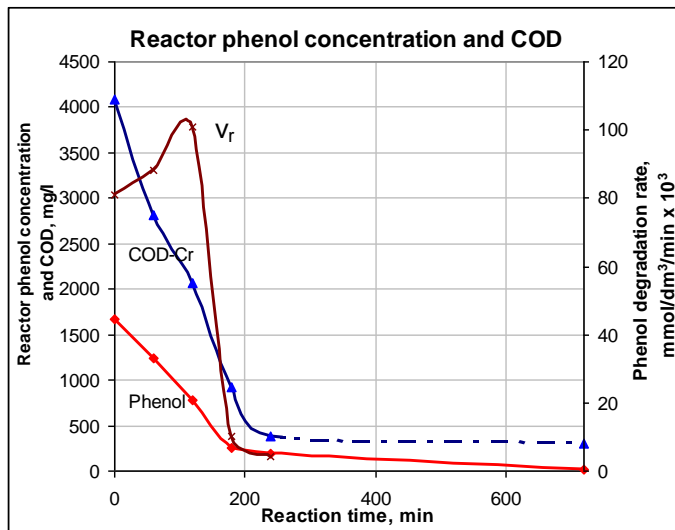
Figure 5b shows some kinetic data obtained on sequencing batch reactor. Not only the reaction time needed to degrade phenol to a certain extent, but also the reaction rate is negatively affected by the increase of phenol concentration. From 1500 mg/l to 2700 mg/l phenol feed concentration (or roughly 900 to 1700 mg/l SBR startup concentration after feed time, lower due to dilution with sludge) the maximum observed reaction rate decrease by almost 50% from 0.16 to 0.09 mmol/(dm<sup>3</sup>·min). As it can be seen, the reaction by-products are biologically further degraded as indicated by the global chemical oxygen demand parameter.



**Fig. 5a.** Aerobic granular sludge SBR performances/stability at increasing influent phenol concentrations

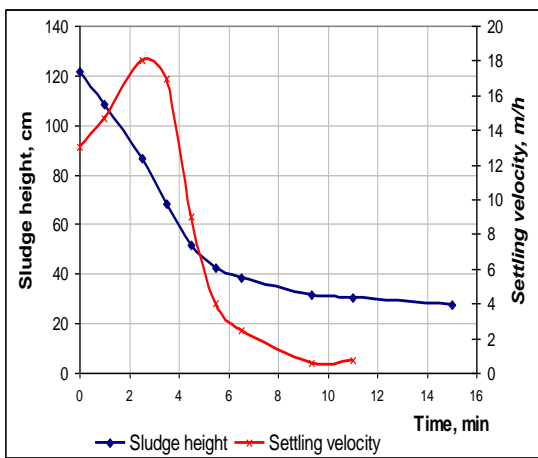


$C_0 = 1500 \text{ mg/l}$

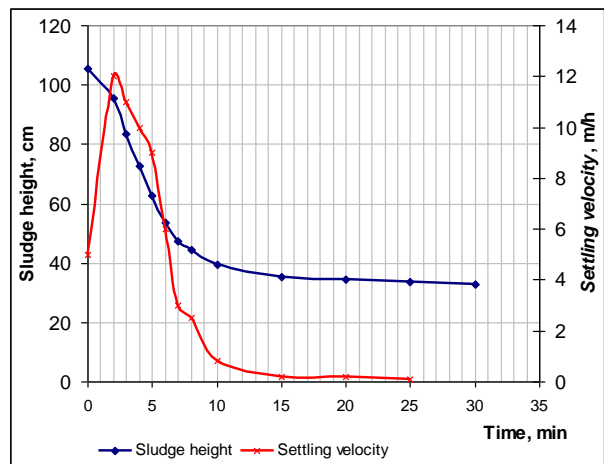


$C_0 = 2700 \text{ mg/l}$

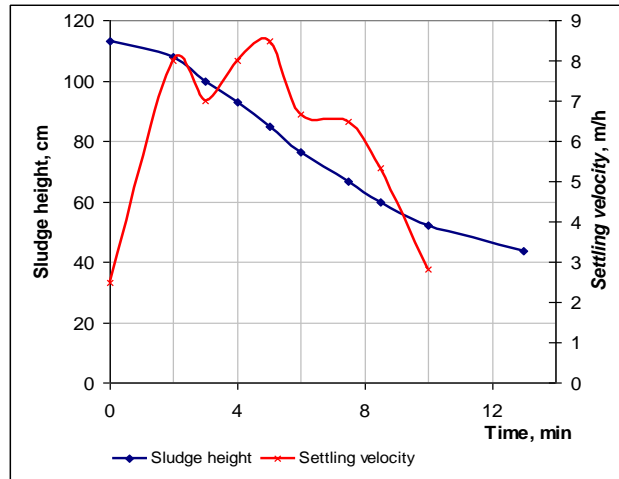
**Fig. 5b.** Phenol degradation by aerobic granular sludge. Some SBR kinetic data ( $C_0$  = phenol feed concentration. Not the same as the startup SBR phenol concentration)



$C_0 = 1000 \text{ mg/l}$



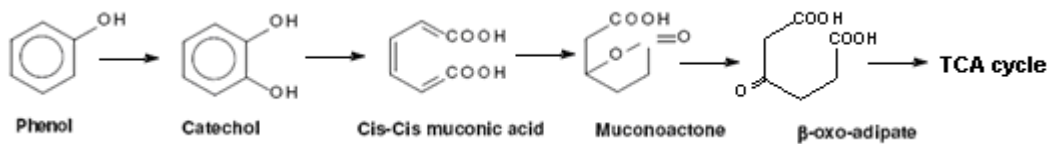
$C_0 = 1500 \text{ mg/l}$



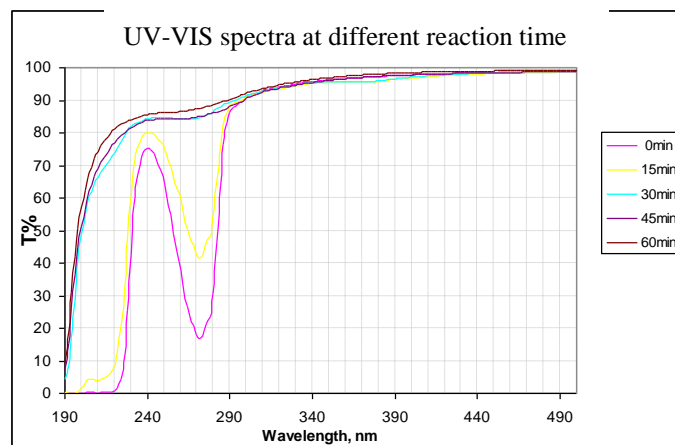
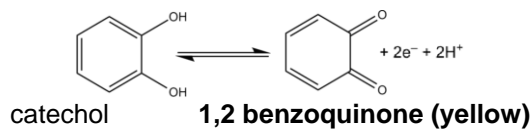
$C_0 = 2700 \text{ mg/l}$

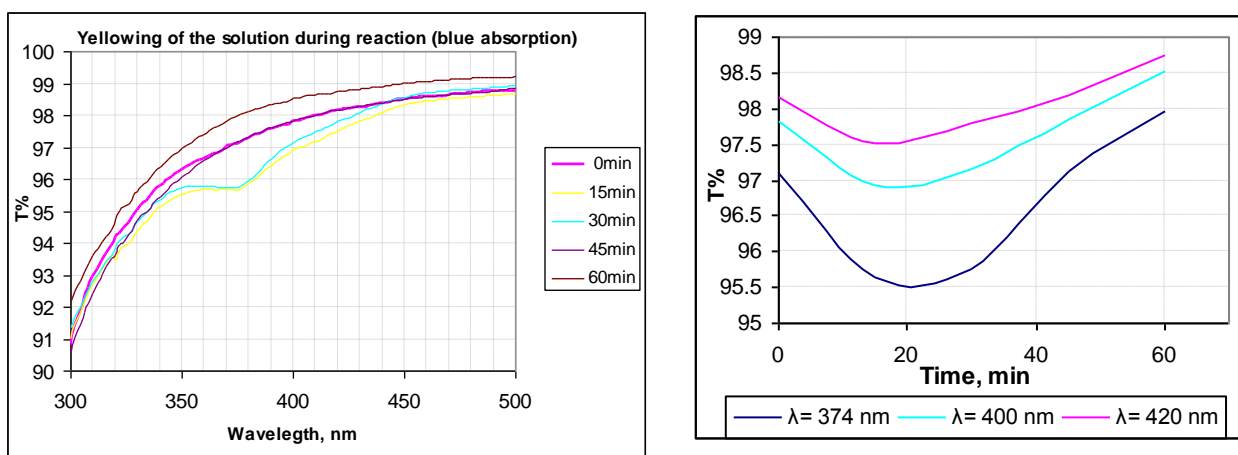
**Fig.6.** Aerobic granular sludge settling curve  
( $C_0 = \text{Influent fenol concentration}$ )

During the first hours of cycle operation, a slight yellowish color is observed in the bioreactor supernatant (fig.7), color which disappears in the next few hours. This may be explained by the formation of the degradation intermediate compound 1,2 benzoquinone (yellow) because aerobically, phenol is first converted to catechol, and subsequently, the catechol is degraded via ortho or meta fission to intermediates of central metabolism (Indu, et. al 2008). The initial ring fission is catalysed by an ortho cleaving enzyme, catechol 1, 2 dioxygenase or by a meta cleaving enzyme catechol 2,3 dioxygenase, where the product of ring fission is a cis-muconic acid for the former and 2-hydrocis muconic semi aldehyde for the latter.



**Ortho pathway of phenol degradation** (TCA = tricarboxylic acid cycle (TCA cycle), the Krebs cycle)





**Fig.7.** Yellowing of the solution during reaction (blue absorption) due to catechol formation and oxidation to benzoquinone

## Conclusions

Acetate-fed granules served as an excellent microbial seed for the development of phenol degrading granules. The aerobic granular sludge showed good stability and adaptability, considering the pollutants dose, removing with high efficiency the toxic concentration of 3g/L and global organic load in less than 8 hours.

The high tolerance of aerobic granules to phenol can be exploited in developing compact high-rate treatment systems for wastewaters loaded with a high concentration of phenol. SBR systems using aerobic granules may prove powerful for removing other inhibitory and toxic organic compounds from high strength industrial wastewaters.

## References

- Allsop PJ, Chisti Y, Moo-Young M. and Sullivan GR. (1993) Dynamics of phenol degradation by *Pseudomonas putida*. *Biotechnol Bioeng*;41:572– 80.
- Bumbac C., Popescu A.M., Pena Leonte E., Dobre D. and Ghita I. (2009), Formation of aerobic granular sludge recent advances and experimental studies, *Environment and Industry International Symposium Proceeding*, vol.1., 132-140
- de Kreuk, N. Kishida and M.C.M. van Loosdrecht, (2007) Aerobic granular sludge – state of the art, *Water Science & Technology* Vol 55 8–9 75–81
- Nair I.C., Jayachandran K. and Shashidhar S., (2008) Biodegradation of phenol , *African Journal of Biotechnology* Vol. 7 (25), pp. 4951-4958,
- Liu Q.S., Liu Y., Show K.Y. and Tay J.H., (2009), Toxicity effect of phenol on aerobic granules, *Environmental Technology*, Vol. 30, No. 1, , 69–74
- Adav S.S., Lee DJ., Show KY. and Tay JH., (2008), Aerobic granular sludge: Recent advances, *Biotechnology advances*, 411-423
- Adav S.S., Lee D.J. and Lai J.Y., (2009) Treating chemical industries influent using aerobic granular sludge: Recent development, *Journal of the Taiwan Institute of Chemical Engineers* 40 333–336
- van Loosdrecht, M.C.M., Pot, M.A. and Heijnen, J.J.. (1997) Importance of bacterial storage polymers in bioprocesses. *Wat. Sci. Technol.*, 35 (1), 41–47.
- Zhang L., Feng X., Zhuc N. and Chena J., (2007), Role of extracellular protein in the formation and stability of aerobic granules, *Enzyme and Microbial Technology* 41 551–557